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BIOLOGICAL EFFECTS OF HONEY*

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Summary

Honeys from eight different floral sources and geographical regions were studied for their biological effects. All eight honeys showed bacteriostatic effects at 100% concentration against three out of four species of micro-organisms; all but one inhibited the fourth species. Rats fed on diets in which honey replaced sucrose or cornstarch in general showed greater weight gains. Stimulation of yeast growth greater than that shown by 1 µg biotin was observed with most of the honeys, with optimum concentration between 10% and 40%. Guinea-pig wrist-joint stiffness was decreased (marginally) only by sweet-clover honey, as well as by ergostanyl acetate, the positive control. Chrysanthemum cuttings dipped in solutions of tupelo, citrus, cotton, or sweet-clover honey developed more roots than cuttings dipped in sugar solution; however, for pyracantha cuttings only tupelo and buckwheat showed such results. Efforts to demonstrate an oestrogenic activity in rats by injection with honey were fruitless. Treatment of the raw honeys with heat to simulate commercially produced honey had little or no adverse effect on rat growth stimulation, and only a partial effect on the stimulation of yeast growth.

Introduction

The literature on honey contains accounts of a variety of responses of living organisms to honey. Some of these are well documented, whereas others are described only once. It was the purpose of the work reported here to examine several of the less-explored activities in somewhat more detail. The reputed activities examined were: yeast growth stimulation, oestrogenic effect, stimulation of rooting of cuttings, the guinea-pig antistiffness factor, the antibacterial effect, and a rat growth effect.

Stimulation of the growth of yeast by inclusion of honey in a "complete" medium has been reported for osmophilic yeasts (Lochhead & Farrell, 1931; Farrell & Lochhead, 1931), and for bread yeast (Murota, Saruno & Ano, 1952). Dingemanse (1938) reported that honey contained variable amounts of a steam-volatile oestrogenic material; Sereno & Montezemolo (1941) found the equivalent of about 22 200 i.u. oestrogenic substance per kg honey (considerably more than reported by Dingemanse), active by oral ingestion or subcutaneous injection.

Oliver (1939) reported that honey stimulated rooting in cuttings. Later, Poma Treccani (1950) in studying the effect of an α-naphthalene-acetic acid on the rooting of vine cuttings noted that the hormone was most effective at 10 ppm when mixed with 10% honey. They both showed that the value of honey lay in its sugar content rather than in the presence of any hormones.

Church (1954) described work in which guinea-pigs showing wrist stiffness, induced by a lack of an "antistiffness factor" in the diet, were treated with honey or wax. Moderate stiffness was cured within a week by daily doses of 500 mg honey

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or beeswax; more severe cases were improved. Addition of unheated honey to the deficient diet generally prevented the appearance of stiffness; boiled honey did not do so.

The antibacterial effect of honey was first described by Dold, Du & Dziao (1937), and confirmed by Prica (1938) and Plachy (1944); it was ascribed to a heat-labile and possibly photosensitive material termed "inhibine". An investigation of this effect was included in the present study, but since this work was completed the nature of inhibine has been clarified by the work of White, Subers and Schepartz (1963); see also White and Subers (1963). Our results, obtained before those of White et al., confirmed the existence of an antibacterial effect, against the following micro-organisms: *Micrococcus flavus, Sarcina lutea, Bacillus cereus* and *B. subtilis.* Since they provide no new insight into the nature of the effect, they will not be further described.

Materials and Methods

Honey samples

The following eight honeys, obtained in the U.S., were examined for the effects described above:

alfalfa (*Medicago sativa*) — sweet clover (*Melilotus*)
tulip tree (*Liriodendron tulipifera*)
orange (*Citrus sinensis*)
tulepo (*Nyssa ogeche*)
eastern buckwheat (*Fagopyrum esculentum*)
sweet clover
eastern fall flower
cotton (*Gossypium hirsutum*).

They are among the honeys reported on by White and Subers (1963). Honey was obtained in 60-pound [27-kg] containers directly from the producers and received no heat treatment. When positive results were obtained with any particular honey, a sample was heat-treated (to simulate the commercially prepared honey) and tested to determine any loss of activity. For most of the studies this treatment consisted of heating the honey to 70°C (equivalent to 160°F used by many commercial honey processors), maintaining this temperature for 30 minutes, and cooling rapidly. Synthetic "honey"—17% water, 32% glucose, 39% fructose, 8% maltose, 2% sucrose, 2% raffinose—provided a negative control for all our studies except those on rat growth.

Antibacterial effect

The pad-plate assay for bacterial inhibition (Kohler & Broquist, 1951) was used. Small circular paper pads were placed on agar medium previously inoculated with the organism. A measured volume of honey, either concentrated (100%) or diluted to 40, 20, 10 or 1% with distilled water, was applied to the pad, and the agar plate was incubated overnight. The diameter of the circular cleared zone surrounding the pad during incubation gave a quantitative estimate of the strength of inhibition. Brain Heart Infusion* was used for assay for *B. cereus* and *B. subtilis* and incubated at 37°; Penassay seed agar* was used for *M. coccus* and *S. lutea,* incubated at 27°.

* Difco Laboratories, Detroit, Michigan, U.S.A.

Rat growth

A solution of 83% sucrose and 17% water mixed with basal diet was used for a negative control. The basal diet consisted of casein 18%, cornstarch [from *Zea mays*] 62·2%, cornstarch–vitamin mixture 10%, salts 4·0%, DL-methionine 0·6%, DL-tryptophan 0·2%, and refined cottonseed oil 5·0%. The cornstarch–vitamin mixture contained the following in 100 g: thiamine 50 mg, riboflavin 50 mg, niacin 100 mg, calcium pantothenate 200 mg, pyridoxine 1 mg, folic acid 2 mg, vitamin B_{12} 0·2 mg, inositol 1 g, choline 15 g. Weekly administration was made by dropper of 2 drops of fortified codliver oil diluted with corn oil to give vitamin A 400 IU, vitamin D 4 IU, menadione 0·04 mg, and α-tocopherol 4 mg. In the test diets cornstarch equivalent to 30% of the diet was replaced with honey, and for the negative control, with the sucrose–water solution.

Groups of 10 male weanling rats of the Sprague-Dawley strain, weighing 32–50 g, were fed *ad libitum* on the test diets containing one of the eight honeys, raw or treated. Initial and weekly weights and weekly food consumption were recorded for 5 weeks, after which the animals were chloroformed and the stomach and intestinal tract removed. The carcases were autoclaved at 15 lb/in² [1·05 kg/cm²] for 4 hours. Water loss was determined after cooling, and the carcases were homogenized in a Waring blender with an amount of water equal to the weight loss. To determine the percentage of solids, 10 g homogenate was mixed with 50 ml 95% ethanol and allowed to stand overnight. Ethanol was removed by evaporation, the sample dried at 105°, and the total solids determined. About 75 ml of 1:1 diethyl ether – petroleum ether (Skelly-solve F) mixture was added to the sample. After standing at room temperature overnight, the ether layer was decanted and discarded, fresh ether added, and the procedure repeated twice. After the third extraction the final trace of ether was evaporated, the sample was dried overnight at 105°, and weighed. Total lipids were considered to be equal to the loss in weight. Carcase protein was determined by micro-Kjeldahl analysis of the ether-extracted oven-dried residue.

Bartlett's test for homogeneity of variance (Bennett & Franklin, 1954) was applied to the data in each of the analytical categories in Tables 1 and 2. Where homogeneity was indicated by the result, pooled values of s were used to calculate t values, using the sucrose diet as a control. In two instances (Table 2, protein and solids) the test indicated inhomogeneity; here the individual s values were used.

Yeast growth

The yeast growth factor was studied by means of the Hertz assay for biotin (Hertz, 1943), with two variations in procedure: (1) all honey samples, both raw and heat-treated, were partially sterilized by heating to 100° at atmospheric pressure and holding at that temperature for 5 minutes to inactivate any rapidly growing organisms; (2) inoculation of the sample was made quantitative by adding two drops of yeast suspension (0·003 mg) from a no.25 hypodermic needle. All samples of diluted honey were filtered through a micropore filter to yield a clear sample. All turbidity readings were made at 660 mμ and recorded as percentage transmission. A correction was made for any background turbidity initially present in the honey. A non-inoculated sample of honey and Hertz medium, incubated with the yeast-inoculated samples, provided a negative control. It was necessary to choose with care the brand of calcium pantothenate used in the yeast growth medium, since several samples showed biotin contamination. In each assay, yeast growth stimulation by

honey was compared with that in a series of tubes containing graded amounts of biotin. The choice was arbitrary, since it was not shown that the stimulation obtained with honey was actually due to the presence of biotin.

Oestrogenic activity

In preparation for the oestrogenic study, vaginal smears were taken from rats each day for a week and stained with haematoxylin and eosin. When normal oestrus was established, the ovaries were removed by general surgical techniques according to Farris and Griffith (1949). Prior to the assay the rats were primed with two subcutaneous injections within a 7-hour period of 1·5 µg of oestrone ($C_{18}H_{22}O_2$) dissolved in 0·1 ml corn oil (Curtis et al., 1944). Vaginal smears were made with dampened cotton swabs at 48, 52½, 56½, and 72 hours after the first injection. All smears from a single animal were placed on different sections of a glass slide and stained for microscopic examination. A smear was considered positive in which the majority of cells were of cornified epithelium. Any rat not responding to oestrone was discarded. For assay of the honeys 0·2 ml of 50% honey replaced the oestrone in each of the two injections. Between assays all animals were primed with a total amount of 3 µg oestrone, and vaginal smears were taken to determine whether the response remained normal. Subsequently, the amount of honey was increased to 0·5 ml of 80% honey in each of two injections.

Guinea-pig wrist-joint anti-stiffness factor

For determinating the wrist-joint anti-stiffness effect of honey, guinea-pigs weighing 200–400 g were fed the pelleted basal diet according to Oleson et al. (1947). In addition each animal received orally 20 mg ascorbic acid three times weekly. The degree of stiffness was determined by the manual procedure of van Wagtendonk and Wulzen (1946), in which scores range from 4, indicating normal flexion, to 1, indicating severe stiffness. In this test the animal's foreleg was extended posteriorly along the body wall, supported from below by the operator's fingers, and held rigid by downward pressure from the operator's thumb on the olecranon process. With his other hand the operator flexed the paw upwards by gentle pressure, and the degree of bending was noted. Subsequently, irradiation of the animals with sunlight was used instead of the vitamin D supplement in the diet. Ergostanyl acetate in corn oil provided a positive control, 50% honey in water was the test substance, and the synthetic "honey" was the negative control. All substances were administered by stomach tube: 5 or 10 µg sterol in 1 ml corn oil, or 5 ml honey or synthetic "honey", daily for 8 days.

Stimulation of plant rooting

To determine the optimum conditions for study of root stimulation by honey, cuttings from new shoots of chrysanthemum and pyracantha plants in the pre-flowering stage were first allowed to stand for 1, 2, 4 or 6 hours (Oliver, 1939) in the test solution, and then planted in sterilized perlite. Honey was used as a 5, 10, 20 or 50% solution (v/v) in water; the positive control was 20 ppm of α-naphthalene acetic acid in water, and the negative control a 50% solution in water of synthetic "honey". Initial environmental conditions—room temperature and humidity (about 32° and 40% respectively)—were discarded in favour of a plastic mist chamber 30 × 30 × 45 cm, which contained a tray of perlite 10 cm deep; this maintained viability in the cuttings. Difficulties with a fungal rot in the root zone were encountered when

honey was compared with that in a series of tubes containing graded amounts of biotin. The choice was arbitrary, since it was not shown that the stimulation obtained with honey was actually due to the presence of biotin.

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soaking times were longer than a few seconds in either honey or control solutions. A dipping period of 5 seconds eliminated this problem.

Results and Discussion

Anti-bacterial effect

All the honeys showed at least a slight inhibition against all four micro-organisms, with the exception of sweet clover honey against *B. subtilis*. Sweet clover – alfalfa and cotton honeys exhibited the strongest effect, inhibiting the growth of both *M. flavus* and *S. lutea* at a 1:10 dilution (10%). White et al. (1963) have nicely characterized the enzymatic nature of this inhibiting substance ("inhibine").

Rat growth

Results of the two tests are shown in Tables 1 and 2. The tests were run at different times and used different litters, with different histories and from different suppliers. In Test 1 only tulip tree and cotton honeys gave a statistically greater weight gain compared with the sucrose control. Two of the groups in Test 1 (treated tulip tree and citrus) showed increased fat deposition at the expense of protein; all but one of the remaining four groups showed a similar tendency, with lower protein values but fat deposition not significantly greater than that for the sucrose control. In Test 2 significantly greater weight gains were obtained with all but one of the honeys than with the sucrose control. The relationship between carcase fat and protein deposited appeared to be the reverse of that in Test 1. With 7 honeys significantly more protein was present, sometimes accompanied by less fat. Heating the honeys brought about negligible changes in their effect on rat growth. A possible explanation of the different responses of the two groups is that on the basis of starting weights, the second group of rats appeared to be several days older than those in the first group. Cage positions were not a factor, and room temperature was carefully controlled throughout the year. An age or litter difference could account for the lower average total weight gains and the lower average terminal weights of the second group.

Further experimental work is obviously required to reach definite conclusions on the effect of honey in rat diet; in any such work the effect of the source and strain of rats should be eliminated.

Yeast growth

Table 3 shows results of experiments in which various concentrations of each of the eight honeys, raw or treated, were incubated with yeast in a complete medium. The values are arbitrarily stated in terms of biotin activity, calculated from a standard growth curve of yeast (0–1000 mμg biotin; transmission values 88%–26% respectively). The negative control, with solutions of 1%–100% of synthetic "honey" showed no stimulation (all values around 91–94% transmission). Since it was desired to determine only the presence or absence of a yeast growth factor and its possible heat lability, and not the chemical composition or actual quantity of the factor, no additional dilution or isolation procedures were attempted. At honey concentrations between 10% and 40%, growth stimulation equivalent to more than 1 μg biotin was obtained with most of the raw honeys, 10% appearing to be the optimum concentration. The growth factor in citrus and tulip-tree honeys did not appear to be extremely heat-labile; however, heated alfalfa – sweet clover, tupelo, buckwheat and

TABLE 1. Effect on rat growth of dietary carbohydrate source[1]. Test 1.

Carbohydrate source	*Terminal weight (g)*	*Weight gain (g)*	*CARCASE COMPOSITION*[2] *Solids (%)*	*Fat (%)*	*Protein (%)*	*Efficiency*[3]
cornstarch (basal)	148·6* ± 13·0	111·1* ± 12·3	31·4 ± 2·2	8·67 ± 1·56	21·0** ± 2·4	40·5* ± 4·0
sucrose (control)	162·6 ± 11·7	125·2 ± 11·3	32·0 ± 1·5	8·06 ± 1·90	23·0 ± 1·4	37·4 ± 3·5
alfalfa – sweet clover	168·7 + 12·1	134·4 ± 11·3	31·9 ± 1·1	8·05 ± 1·11	22·2 + 1·5	38·6 ± 1·7
treated alfalfa – sweet clover	162·1 ± 21·1	124·8 ± 19·8	31·8 ± 1·3	8·37 ± 1·74	21·1* ± 1·3	35·2 ± 3·8
tulip tree	175·8* ± 8·6	138·6* ± 7·7	31·7 ± 2·6	9·61 ± 3·04	20·5** ± 1·2	37·3 ± 3·6
treated tulip tree	174·7 ± 12·8	135·9 ± 10·6	32·8 ± 2·1	10·18* ± 2·07	20·1** ± 1·7	35·0 ± 1·9
citrus	172·5 ± 13·4	136·0 ± 12·6	32·9 ± 2·5	10·1* ± 1·90	20·0** ± 1·0	37·1 ± 2·1
treated citrus	169·1 ± 10·6	132·5 ± 10·3	31·9 ± 2·3	9·59 ± 1·24	20·8** ± 1·0	33·9* ± 2·7
cotton	175·9* ± 13·0	139·4* ± 11·1	32·9 ± 1·3	8·75 ± 1·41	20·5** ± 1·0	37·4 ± 2·1
treated cotton	168·7 ± 15·2	132·3 ± 15·5	32·6 ± 1·8	8·27 ± 1·37	20·1** ± 2·0	34·4* ± 4·1

[1] mean ± SD of 10 animals per diet group

[2] calculated on basis of homogenized gutted carcase

[3] gain in g per 100 g food consumed for final 4 weeks

* significantly different from sucrose control at 5% level

** significantly different from sucrose control at 1% level

TABLE 1. Effect on rat growth of dietary carbohydrate source[1]. Test 1.

Carbohydrate source	*Terminal weight (g)*	*Weight gain (g)*	*CARCASE COMPOSITION*[2]			*Efficiency*[3]
			Solids (%)	*Fat (%)*	*Protein (%)*	
cornstarch (basal)	148·6* ± 13·0	111·1* ± 12·3	31·4 ± 2·2	8·67 ± 1·56	21·0** ± 2·4	40·5* ± 4·0
sucrose (control)	162·6 ± 11·7	125·2 ± 11·3	32·0 ± 1·5	8·06 ± 1·90	23·0 ± 1·4	37·4 ± 3·5
alfalfa – sweet clover	168·7 + 12·1	134·4 ± 11·3	31·9 ± 1·1	8·05 ± 1·11	22·2 + 1·5	38·6 ± 1·7
treated alfalfa – sweet clover	162·1 ± 21·1	124·8 ± 19·8	31·8 ± 1·3	8·37 ± 1·74	21·1* ± 1·3	35·2 ± 3·8
tulip tree	175·8* ± 8·6	138·6* ± 7·7	31·7 ± 2·6	9·61 ± 3·04	20·5** ± 1·2	37·3 ± 3·6
treated tulip tree	174·7 ± 12·8	135·9 ± 10·6	32·8 ± 2·1	10·18* ± 2·07	20·1** ± 1·7	35·0 ± 1·9
citrus	172·5 ± 13·4	136·0 ± 12·6	32·9 ± 2·5	10·1* ± 1·90	20·0** ± 1·0	37·1 ± 2·1
treated citrus	169·1 ± 10·6	132·5 ± 10·3	31·9 ± 2·3	9·59 ± 1·24	20·8** ± 1·0	33·9* ± 2·7
cotton	175·9* ± 13·0	139·4* ± 11·1	32·9 ± 1·3	8·75 ± 1·41	20·5** ± 1·0	37·4 ± 2·1
treated cotton	168·7 ± 15·2	132·3 ± 15·5	32·6 ± 1·8	8·27 ± 1·37	20·1** ± 2·0	34·4* ± 4·1

[1] mean ± SD of 10 animals per diet group

[2] calculated on basis of homogenized gutted carcase

[3] gain in g per 100 g food consumed for final 4 weeks

* significantly different from sucrose control at 5% level

** significantly different from sucrose control at 1% level

TABLE 2. Effect on rat growth of dietary carbohydrate source[1]. Test 2.

Carbohydrate source	*Terminal weight (g)*	*Weight gain (g)*	*CARCASE COMPOSITION*[2]			*Efficiency*[3]
			Solids (%)	*Fat (%)*	*Protein (%)*	
cornstarch (basal)	123·2* ± 11·2	73·5* ± 8·5	33·7 ± 4·5	10·7 ± 2·38	23·6* ± 5·9	22·1** ± 2·2
sucrose (control)	134·1 ± 15·7	85·9 ± 13·6	35·7 ± 1·2	11·1 ± 1·27	19·4 ± 1·1	27·3 ± 2·6
tupelo	144·9 ± 8·4	94·0 ± 7·1	34·2* ± 1·4	9·36* ± 1·81	20·0 ± 1·1	29·0 ± 2·4
treated tupelo	151·8** ± 11·2	101·3** ± 11·5	34·1** ± 0·6	9·81 ± 1·18	20·1 ± 0·7	31·0** ± 2·8
buckwheat	150·5** ± 8·5	102·3** ± 7·2	34·8 ± 1·3	10·34 ± 2·06	21·9** ± 1·5	32·7** ± 3·6
treated buckwheat	154·2** ± 10·0	105·0** ± 7·1	34·2** ± 1·1	9·95 ± 2·06	21·0** ± 1·3	34·0** ± 3·7
sweet clover	150·2** ± 15·4	102·1** ± 14.8	33·3** ± 1·4	9·39* ± 1·84	21·8** ± 1·4	32·9** ± 3·6
treated sweet clover	159·1** ± 7·7	110·1** ± 8·6	34·5* ± 0·8	10·84 ± 1·32	22·4** ± 1·4	33·4** ± 2·7
fall flower	153·9** ± 13·2	105·5** ± 11·6	33·2** ± 0·6	9·10* ± 1·20	23·4** ± 1·3	33·0** ± 2·7
treated fall flower	147·9* ± 14·8	99·4* ± 13·5	32·6** ± 3·2	9·34* ± 1·57	22·1** ± 2·2	32·2** ± 2·7

[1] mean ± SD of 10 animals per diet group

[2] calculated on basis of homogenized gutted carcase

[3] gain in g per 100 g food consumed for final 4 weeks

* significantly different from sucrose control at 5% level

** significantly different from sucrose control at 1% level

TABLE 3. Stimulation of yeast growth by various raw and heat-treated honeys.

Honey concentration	*1%*	*10%*	*20%*	*40%*	*80%*
	Activity, arbitrarily expressed in terms of mμg biotin, on the basis of a standard curve				
citrus	1·3	>1000	>1000	100	0
treated citrus	0	>1000	>1000	>1000	0
alfalfa – sweet clover	7·0	25	20	15	0
treated alfalfa – sweet clover	0	2·8	3·3	3·0	0
tupelo	0·8	>1000	>1000	>1000	0
treated tupelo	0	>1000	2·3	1·3	0
tulip tree	1·7	>1000	>1000	>1000	0
treated tulip tree	0	>1000	1000	1000	0
buckwheat	100	>1000	>1000	>1000	0
treated buckwheat	0	>1000	2·3	1·4	0
cotton	4·4	>1000	>1000	>1000	0
treated cotton	0	>1000	4·5	2·5	0
fall flower	0·3	3·7	2·3	1·2	0
treated fall flower	0	>1000	>1000	1000	0
sweet clover	0·6	3·3	4·7	3·1	0
treated sweet clover	0	2	3	1·7	0

cotton honeys showed large decreases in stimulation except at the 10% concentration. The fact that fall-flower honey showed little stimulation until after heat treatment could be explained by the presence of a heat-labile yeast growth inhibitor.

Oestrogenic effect

The results of the oestrogenic study were completely negative for all eight honeys. With ten rats per group, and injection of 1·12 g honey, none of the rats responded with a positive vaginal smear, although each responded to injections of oestrone. These findings do not agree with the report of Serono and Montezemolo (1941) that 0·9 g of honey contained 20 IU of oestrus-producing substances. The presence or absence of this factor may depend on floral source or geographical origin. The method reported here will detect 1·5 μg of oestrone; if the factor was present in the honeys tested, it must be in amounts less than $1{\cdot}3 \times 10^{-4}$%.

Wrist stiffness

Development of wrist-stiffness in guinea-pigs to be tested with honey was hindered apparently by the presence in the diet of an isomer of ergosterol or one of its derivatives. Even after four months on the deficiency-producing diet the animals failed to show the stiffness syndrome. However, when vitamin D supplement was omitted from the diet and the animals were exposed to sunlight, they showed signs of wrist-stiffness within four weeks.

Table 4 shows the results for each animal in 13 groups. All treatments were given by stomach tube except for the first group; all honey treatments were made with 50% aqueous solutions. Analysis of the data indicated that between 12 and 24 animals would be required for each group to obtain statistically significant results. One honey gave results similar to the two controls, and when it was heated the

TABLE 4. Effect of honey on guinea-pig wrist stiffness.

The first column for each group gives the initial index for each animal (on the scale 4 = normal flexion, 1 = severe stiffness). The second column gives the final index, and the third column the net change (+ = improvement, — = deterioration). Treatments are ranked in decreasing order of apparent effectiveness (algebraic sum of entries in third column).

*Ergostanyl acetate**			*Sweet clover*			*Ergostanyl acetate*		
3	4	+1	2	3	+1	2	1	−1
3	4	+1	1	2	+1	3	4	+1
2	3	+1	2	4	+2	1	2	+1
2	4	+2	3	4	+1	1	2	+1
			2	1	−1	3	4	+1
			2	3	+1	3	4	+1

Buckwheat			*Synthetic "honey"*			*Citrus*			*Treated buckwheat*		
3	3	0	3	3	0	3	3	0	3	2	−1
3	4	+1	3	4	+1	3	4	+1	1	1	0
2	4	+2	3	4	+1	3	3	0	3	4	+1
1	1	0	3	4	+1	2	4	+2	2	3	+1
3	3	0	2	2	0	3	3	0	2	2	−1
3	4	+1	2	1	−1	1	1	0	2	1	−1

Fall flower			*Alfalfa – sweet clover*			*Treated sweet clover*		
2	3	+1	3	3	0	3	3	0
2	2	0	3	2	−1	3	3	0
2	2	0	2	2	0	2	2	0
3	2	−1	2	2	0	2	3	+1
3	3	0	3	3	0	3	2	−1
1	1	0	2	3	+1	3	3	0

Tupelo			*Cotton*			*Tulip tree*		
2	2	0	1	1	0	2	1	−1
3	3	0	3	3	0	1	1	0
1	1	0	3	3	0	2	2	0
1	2	+1	2	2	0	3	2	−1
3	3	0	2	1	−1	3	3	0
2	2	0	3	2	−1	2	2	0

* 5 μg given by injection; all other groups treated with 10 μg by stomach tube

effect was lost. Others appeared to have less effect, but in the absence of statistical significance, no conclusions are warranted.

Root growth

Results of the experiments are presented in Tables 5 and 6, in terms of the average number of roots formed per cutting. Under the experimental conditions used, chrysanthemum cuttings required 3–4 weeks to develop roots, and pyracantha cuttings required 6–8 weeks. The control cuttings (treated with synthetic "honey" and α-naphthalene acetic acid) showed great variations in the numbers of roots produced, giving large standard deviations. It is possible that a longer exposure of the cuttings to the test solutions might decrease these variations between cuttings receiving the same treatment.

The *t*-test was applied with the results seen in Tables 5 and 6. With chrysanthemum cuttings, significant increases in rooting (compared with synthetic "honey"

TABLE 5. Effect of honey on chrysanthemum rooting. Average number of roots per cutting.

Honey	*Honey concentration*				*Synthetic honey, 50%*	*α-Naphthalene-acetic acid 20 ppm*
	5%	*10%*	*20%*	*50%*		
tupelo	31·1* ± 8·8	30·9* ± 9·9	27·2 ± 10·6	26·4 ± 9·0	23·6 ± 11·5	33·8** ± 9·8
buckwheat	23·8 ± 7·3	23·0* ± 7·2	28·6 ± 6·6	27·0 ± 6·4	27·8 ± 7·8	27·6 ± 7·0
sweet clover	33·7 ± 6·6	26·9 ± 10·2	34·2 ± 9·5	29·2 ± 12·4	32·8 ± 10·9	25·4* ± 10·4
tulip tree	21·2 ± 7·5	22·1 ± 5·8	21·8 ± 9·3	24·4 ± 6·8	25·6 ± 12·2	26·9 ± 12·6
alfalfa – sweet clover	29·4* ± 9·2	19·2 ± 9·8	20·4 ± 7·6	22·4 ± 11·2	21·3 ± 12·0	23·9 ± 11·0
cotton	29·0 ± 9·6	24·8 ± 7·1	36·4* ± 8·8	29·8 ± 7·2	28·8 ± 13·3	29·6 ± 7·1
citrus	24·4 ± 6·0	23·8 ± 6·3	25·3* ± 6·1	21·0 ± 5·9	21·0 ± 5·9	20·0 ± 5·0
fall flower	28·1 ± 10·3	28·2 ± 8·2	28·2 ± 7·6	30·2 ± 5·7	27·8 ± 8·7	28·9 ± 5·9

* significantly different from synthetic "honey" control at the 5% level

** significantly different from synthetic "honey" control at the 1% level

TABLE 6. Effect of honey on pyracantha rooting. Average number of roots per cutting.

Honey	*Honey concentration*				*Synthetic honey, 50%*	*α-Naphthalene-acetic acid 20 ppm*
	5%	*10%*	*20%*	*50%*		
tupelo	5·2* ± 3·4	4·5* ± 2·7	4·0 ± 2·1	4·3 ± 2·0	3·0 ± 2·2	3·5 ± 1·9
buckwheat	4·1 ± 3·1	5·2* ± 2·3	4·8 ± 2·1	4·0 ± 2·0	3·7 ± 2·2	3·3 ± 3·1
sweet clover	4·1 ± 2·4	3·8 ± 1·8	4·2 ± 2·8	4·2 ± 2·2	3·6 ± 1·7	4·2 ± 2·4
tulip tree	3·0 ± 2·0	3·6 ± 1·5	3·2 ± 1·5	3·2 ± 1·4	3·5 ± 1·6	2·8 ± 2·3
alfalfa – sweet clover	3·4 ± 1·5	3·0 ± 1·9	3·0 ± 1·4	3·0 ± 1·4	2·7 ± 1·3	3·0 ± 2·3
cotton	4·2 ± 3·1	3·8 ± 2·5	4·1 ± 1·7	3·8 ± 2·7	3·8 ± 1·8	3·5 ± 1·5
citrus	3·6 ± 1·3	2·8* ± 1·2	3·0 ± 1·9	4·4 ± 2·7	4·0 ± 2·3	3·6 ± 1·5

* significantly different from synthetic "honey" control at the 5% level

controls) were shown with one honey at 5% and 10%, and with three other honeys at one concentration only. One honey at 10% showed a significant decrease. With pyracantha cuttings results were rather similar.

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